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Tools and Technology

Assessing Chemical Control of Earthworms at Airports

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ABSTRACT Earthworms originating from Europe (e.g., *Lumbricus* spp., *Aporrectodea* spp.), which are common in the United States and southern Canada can create hazardous conditions at airports by attracting birds that pose a threat to aircraft. These nonnative earthworms are also considered pests on golf courses and sports fields, as well as having detrimental effects on temperate boreal forests. No toxicants or repellents are currently registered for earthworm control in the United States. Our purpose was to identify products that could be used to repel or suppress nonnative earthworms on airports or other managed sites where they pose a hazard or nuisance. We conducted experiments on the National Aeronautics and Space Administration's Plum Brook Station, Ohio, USA, and at an area about 15 km south of this site, during 2007–2013. We hypothesized that either abrasiveness or extreme low or high pH levels would be characteristic of an efficacious repellent. Ammonium sulfate fertilizer (an acidic treatment) repelled *Lumbricus terrestris* in choice tests. However, 6 applications of ammonium sulfate over 2 years only reduced the field density of *Aporrectodea* spp., and not *Lumbricus* spp. Application of tea-seed cake pellets (TSP), a saponin-rich byproduct of tea oil (*Camellia oleifera*) manufacture that causes earthworms to come to the surface and desiccate, temporarily reduced densities of both *Lumbricus* and *Aporrectodea* spp. in field plots. However, several applications per year would probably be needed for sustained control. Ring-billed gulls (*Larus delawarensis*) showed no apparent adverse effects over a 48 hour period from feeding on TSP-killed earthworms. We discuss potential value and limitations of TSP and other methods for managing earthworms to reduce airport bird-strike hazards.

KEY WORDS airport, *Aporrectodea*, bird–aircraft collisions, *Camellia oleifera*, earthworm control, fertilizer, *Lumbricus*, tea seed, triterpene saponins.

Earthworms (Lumbricidae) are generally regarded as beneficial because they improve soil structure, fertility, and productivity through their burrowing activity and breakdown of organic matter, and aid in nutrient cycling for plants (Edwards and Bohlen 1996). In North America, native species of earthworms were removed from areas of glacial activity, (Reynolds 1994, Hendrix and Bohlen 2002). Now, in those same areas, European Lumbricidae, introduced via soils associated with plants transported from abroad and dry ballast, are the nonnative worms most often found (Hendrix and Bohlen 2002, Keller et al. 2007).

Despite benefits to soil structure and plants, nonnative earthworms may also pose hazards to airport operations. When earthworms crawl onto runways and aprons after rain events, they can attract gulls (Laridae), starlings (*Sturnus vulgaris*), or other birds that might subsequently be struck by aircraft (Kirkham and Morris 1979; Feare 1984; Hillström et al. 1994; Dolbeer et al. 2000, 2014; DeVault et al. 2011). For example, during a 30 minute interval following a rain storm at Calgary International Airport, Canada, a Boeing 737 and an Airbus 319 struck gulls that were feeding on earthworms and incurred significant damage during takeoff (Air Safety Week 2004; R. Dolbeer, United States Department of Agriculture, personal communication). Additionally, earthworms on runways can create a safety hazard because of a loss of friction for moving aircraft (United Press International 1972, Nichols 2013). Current runway designs do not accommodate physical barriers to earthworms (Federal Aviation Administration 2007). Further, even a design that impeded earthworm access to a runway might not prevent their accumulation, and associated bird activity, in areas bordering runways and aprons after rains.

Aside from hazards to aviation, when populations are high, earthworms can become pests on recreation areas where their castings either impede recreational pursuits or cause turf

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damage (Kirby and Baker 1995, Edwards and Bohlen 1996, Williamson and Hong 2004, Potter et al. 2013). Nonnative earthworms also have detrimental effects on forest ecosystems by changing the characteristics of the forest floor through litter removal, alteration of soil horizons, and influencing soil geochemical processes (Bohlen et al. 2004 *a*, *b*; Hale et al. 2006; Keller et al. 2007; Loss and Blair 2011; Loss et al. 2012).

Despite their nonnative status and recognition as pests in various situations, no chemical products are currently registered for earthworm control in the United States. Cultural control (e.g., grass clipping removal), application of acidifying fertilizers, or off-label use of pesticides can often provide partial suppression of earthworms or their casts (reviews, Kirby and Baker 1995, Potter et al. 2013). Williamson and Hong (2004), for example, found that application of abrasive aggregates reduced casts on golf course fairways, but efficacy declined within months as the material became incorporated into the soil. Reducing soil pH by use of acidifying fertilizers over multiple years has also shown a reduction in earthworm numbers, but results vary by species and soil characteristics (Slater 1954, Potter et al. 1985, Ma et al. 1990).

A recently developed and potential earthworm control tool may be tea-seed cake pellets (TSP), a byproduct of tea oil (*Camellia oleifera*) manufacture. Tea-seed cake pellets contain triterpene saponins, natural detergents that are used in shampoos and soaps, as emulsifiers, and for other purposes (Potter et al. 2010). Saponins, as a group, have been shown to have anti-inflammatory and anti-oxidant properties (Sur et al. 2001, Chattopadhyay et al. 2004, Lee and Yen 2006), gastroprotective effects in rats (*Rattus* spp.; Morikawa et al. 2006), and anti-carcinogenetic properties (Rao and Sung 1995, Ghosh et al. 2006). Triterpene saponins irritate the mucus membranes of molluscs (Mollusca) and earthworms (San Martín et al. 2008, Potter et al. 2010). Crude TSP or products containing tea-seed saponins are used as organic molluscicides in some parts of the world (e.g., Yang et al. 2006). Tea-seed cake pellets were recently shown to expel earthworms from turfgrass, causing them to desiccate and die on the surface, which reduced casts in golf-course settings (Potter et al. 2010). The same rates of TSP that expelled earthworms did not affect black cutworms (*Agrotis ipsilon*), scarab grubs (*Cyclocephala* spp.), or any of the major taxa of soil microarthropods (Potter et al. 2010). A specialty fertilizer (Early Bird™; Ocean Organics, Waldoboro, ME) containing tea-seed meal or extract has been marketed as a natural fertilizer. However, it is unknown whether TSP or tea-seed saponins affect both common genera of earthworms found on airports (*Lumbricus* and *Aporrectodea* spp.; O'Neal and Forbes 1987), or whether birds (e.g., ring-billed gulls [*Larus delawarensis*], a species frequently struck by aircraft; Dolbeer et al. 2014) that consume earthworms expelled by those substances will suffer any ill effects.

The objectives of this study were as follows: 1) to quantify earthworm behavioral response to direct exposure to candidate repellents in a simulated runway scenario; 2) evaluate earthworm population response to the most promising repellents in a simulated airport application; 3) test effectiveness of TSP for suppressing densities of both genera of earthworms that predominate on U.S. airports; and 4) assess feeding response of gulls to TSP-expelled earthworms and any short-term malaise resulting from gull consumption of them. Our long-term goal is to find methods to reduce the number of earthworms that emerge onto airport runways, taxiways, or other hard surfaces where they pose slippage or traction issues for aircraft, as well as increasing risk of bird–aircraft collisions.

STUDY AREA

We conducted experiments on the 2,200 ha National Aeronautics and Space Administration's Plum Brook Station (PBS; Erie County, OH; 41°22′N, 82°41′W) and at an area about 15 km south of PBS. Landscape types within PBS included old field and grasslands that we used for a simulated airport experiment. The test area received about 102 cm annual precipitation and had a frost-free period of about 140–180 days.

We selected 3 candidate repellents based on a literature review, anecdotal reports, and previous unpublished experiments (Table 1). The simulated runway experiments were done in an enclosed building with windows covered to prevent sunlight from striking test areas. All lighting came from overhead fluorescent lights. Earthworms (adult *Lumbricus terrestris*) were purchased from American Rod and Gun™ (Springfield, MO). All procedures were approved by the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, Institutional Animal Care and Use Committee (QA-1294, -1915, -1966).

METHODS

Simulated Runway Experiments

Ideally, a simulated runway design would require worms to move from soil through a treatment to the simulated runway, but the conditions that drive worms from soil during rain events (e.g., water infiltration and removal of oxygen) remain

Table 1. List of substances and their active ingredients used in controlled tests of potential earthworm repellents. We conducted experiments on the National Aeronautics and Space Administration's Plum Brook Station, Ohio, USA, during 2007–2011.

			Test used in…	
Treatment	Substance type	Active ingredient	Simulated runway	Field
Coal slag	Abrasive	Crushed slag	X	
21-0-0 ammonium sulfate fertilizer	Chemical	$(NH_4)_2SO_4$	X	X
Deer repellent	Chemical	Capsaicin	X	

unclear (Edwards and Bohlen 1996). Therefore, this experiment quantified behavioral effects of earthworms moving from an undesirable situation (i.e., a well-lighted, white, hard surface) to a treated or control soil environment. We constructed 2 identical plywood tables covered with white Formica® (Formica Group-Fletcher Building, Auckland, New Zealand)—1 to serve as a control arena and the other as the treatment arena (Fig. 1). Each table was 91 cm wide, 175 cm long, and 13 cm deep. The central area of each table (i.e., our simulated runway; 91 cm wide and 61 cm long) was 7 cm above either end, thus providing 7 cm of soil depth in either end of the table. The ends of each table were divided in half by a transparent Plexiglas® sheet placed perpendicular to the center area such that it formed a barrier from the bottom of the table to the soil surface (Fig. 1). As a result, each table contained 4 quadrants of soil, designated 1–4; each was 59 cm long, 45 cm wide, and 7 cm deep. The Plexiglas sheet served to prevent earthworms from subterranean movement from one quadrant to the next, but did not prevent surface movement of earthworms, which we would be able to observe.

For each replication on the treatment table, we randomly selected a treated quadrant, thus establishing the remaining quadrants as treated and control, and on a diagonal from each other. The control table quadrants did not receive any substances (other than water; see below) and were treated similarly. We filled quadrants with topsoil purchased from a local nursery. We placed control and treatment tables side by side oriented in an east–west direction for each experiment. Table positions were switched after each replication to avoid possible bias, including electromagnetic cues. We recorded ambient light intensity at the center of each table using a Li-Cor LI-250 Light Meter and LI-190SA Quantum Sensor (Lincoln, NE). Data could not be normalized, so we used the Wilcoxon rank-sum test at a significance of $P < 0.05$ to compare light intensity between tables.

Figure 1. We conducted experiments on the National Aeronautics and Space Administration's Plum Brook Station, Ohio, USA, during 2007–2011 to identify products that could be used to repel or suppress nonnative earthworms on airports or other managed sites. Simulated runway experimental apparatus showing 1 of 2 tables with a central area from which earthworms would move into soil quadrants. One table contained 2 quadrants of treated and 2 quadrants of control soils, while the second table served as a control table.

We placed a candidate repellent (Table 1) on the 2 designated treated quadrants (treatment table) and then moistened all 4 quadrants in each table with deionized water (150 mL each). Repellents were evenly spread across each treated quadrant. We placed 16 randomly selected earthworms along the edge of the center area of each table such that each quadrant had 4 individuals evenly spaced along the quadrant edge but not in the quadrant (Fig. 1). We would then take observations for both tables for the following 60 minute. The earthworms were allowed to enter and leave quadrants (i.e., re-enter the central arena and remain, return to a former quadrant, or enter a new quadrant) for 60 minute, after which we noted the final location of each earthworm, but we used only those earthworms in quadrants to compare use of treated or control quadrants. We conducted 5 experiments; each replicated 6 times. We used a square-root transformation to normalize the data. To test the possibility that earthworm movement between quadrants on the treatment table could introduce chemical residues to untreated quadrants, we used a paired t-test (significance was assessed at $P < 0.05$) to compare use of treated and untreated quadrants on the treatment table. We also compared similar quadrants on the control table, such that the mean use of quadrants 1 and 4 (diagonally opposed) was compared with the mean use for quadrants 2 and 3. Given that the control table was independent of any soil treatment, the quadrant use ratio from this table served as the expected distribution of earthworms. We compared the mean of the ratios of worm use of quadrants between the treated and control tables using a 2-sample t-test with 5 degrees of freedom (significance was assessed at $P < 0.05$).

We conducted a second simulated runway experiment with ammonium sulfate to test for longevity of effect by counting the number of earthworm holes visible at the soil surface. On both the treatment and control tables, we placed 12 earthworms evenly within each quadrant (48 earthworms total) in soil approximately 2.5 cm deep that had been watered to moderate soil moisture. We applied ammonium sulfate (23 g, the equivalent of 893 kg fertilizer/ha or 180 kg N/ha) and 500 mL of deionized water to each treated quadrant. Control table quadrants received only the 500 mL of deionized water. After approximately 24 hr, we recorded the number of earthworm holes visible at the soil surface, by quadrant; then we covered all holes with existing soil and added 500 mL of deionized water (both tables) to each quadrant. We repeated that procedure for 4 days, with 6 independent replications completed for treatment and control tables. We computed the ratio of holes on the treated table between treated and untreated quadrants, and between quadrants on the control table, for each day. Data were normally distributed for both tables, so we compared results by a repeated-measures analysis of variance with hole-ratio as the dependent variable, replication (i.e., independent groups of 12 earthworms) as subject factor, table as the between-subject factor, and day the within-subject factor (significance was assessed at $P < 0.05$).

Simulated Airport Applications

We further tested ammonium sulfate fertilizer as a candidate repellent in a simulated airport application by applying the fertilizer and subsequently comparing the number of earthworms found in treated and control areas. We proceeded on the assumption that a reduction in earthworm numbers would result in fewer earthworms emerging onto the surface during rain events. We selected 3 areas at the PBS facility based on U.S. Department of Agriculture/Natural Resources Conservation Service soil maps of Erie County, Ohio (Robbins and Martin 1998). Site 1 consisted of Udorthents loamy soil (0–6% slope) in which the original soil had been disturbed and covered with a loamy fill material. Site 2 consisted of about equal areas of Dunbridge loamy sand and Oakville loamy fine sand. Both of these soil series were on a maximum of 6% slope with pH of 6.3 (Oakville) and 7.0 (Dunbridge). Site 3 was approximately 75% Millsdale silty clay loam and 25% Condit silt loam. Both have a maximum of 1% slope and pH of 6.9–7.1.

At each site, we marked 12 plots (5×5 m), separated from one another by ≥ 5 m. The plots were sampled in May 2010 by randomly tossing a square frame (0.125 m^2) into each plot and applying mustard-based expellant (Gunn 1992, Hale 2007) to verify earthworm presence. The mustard mix used consisted of 40 g of dry mustard powder mixed with about 4 L of tap water. We applied this mix over a 15 min period to sample all plots. The active ingredient in mustard powder—allyl isothiocyanate—dissipates as a gas and has no apparent effect on worm behavior or density after approximately 30 minute (T. W. Seamans, unpublished data). We randomly assigned 6 plots at each location to receive the equivalent of 180 kg of ammonium sulfate/ha, yielding 18 treated and 18 control plots.

In 2010 and again in 2011, we applied 3 treatments of ammonium sulfate at 8 week intervals and we sampled plots 6 weeks after treatment, as described above. We identified earthworms to genus (Hale 2007), counted all, and in 2011 weighed the samples. Only data from the pretreatment period and the last sampling period are reported to represent the cumulative effects of the fertilizer treatment over the 2 year period. Data could not be normalized, so we used the Wilcoxon rank-sum test (significance was assessed at $P < 0.05$) to compare earthworm numbers and mass between treated and control plots.

We measured soil pH with a Spectrum Technologies (Aurora, IL) pH meter 4–6 weeks after applying ammonium sulfate for treatments 1 through 5, but not after application 6. The pH data could not be normalized, so we used the Wilcoxon rank-sum test to compare pH data between treated and control plots following the fifth measurement only (significance was assessed at $P < 0.05$).

Gull Exposure to TSP Application

As noted above, Potter et al. (2010) demonstrated the effectiveness of TSP in managing *Aporrectodea* spp. in turf. However, whether birds would eat TSP-treated earthworms and whether the potential toxicity would affect them was not previously examined. Therefore we exposed ring-billed gulls to treated earthworms to determine whether they would consume them in similar amounts as untreated worms and still maintain body weight. We exposed 300 *Lumbricus terrestris* to TSP using one of the tables described above. We placed 150 earthworms on each soil area and allowed them to burrow in. We then applied TSP, in the form of granular Early Bird fertilizer at the recommended rate of 2.93 kg/100 m^2 to both ends of the table and watered the fertilizer into the soil until saturation of the soil. During the following hour, we removed all earthworms that came to the surface then dug into the soil and removed the remaining ones that had been exposed to the TSP leachate. These TSP-treated earthworms and an equivalent number of untreated ones were used to quantify potential toxicity to ring-billed gulls under the conditions of a simulated TSP application. The time between exposure to the TSP leachate and time to feeding ranged from 15 to 60 minutes, and there is no evidence that this time lag would change the amount of TSP present on each earthworm (Potter et al. 2010). The period between removal of maintenance food from the gulls (see below) and earthworm placement was 5 hr.

We captured 20 ring-billed gulls at a landfill in northern Ohio, leg-banded each, sexed individuals via external measurements (Ryder 1978), and recorded their initial body weights. The birds were placed in an outdoor aviary in individual cages ($1.2 \times 1.2 \times 1.8$ m) and fed a diet of fish, pet food, bread, and earthworms with fresh water provided *ad libitum*. We tracked the amount of food consumed by each bird throughout 12 days of captivity by counting the pieces of food eaten to ensure that birds were feeding regularly. One day before exposing gulls to TSP-treated worms, we removed all food from the pens at dawn. Each gull was offered 20 untreated earthworms about 2 hr after food removal to determine how many it would consume in 90 minute. We removed any remaining earthworms after 90 minute and then provided the maintenance diet.

The aforementioned procedure was repeated on the day of the experiment, except that gulls had no food for 5 hr prior to presentation of the earthworms (as noted above). We provided 10 randomly selected gulls 20 TSP-treated worms each; the remaining 10 gulls each received 20 untreated worms. Both treatment and control groups contained 5 male and 5 female gulls. We observed gulls from about 3 m away as they fed to see whether there were any overt reactions to treated earthworms. We also observed the birds for any obvious changes in feeding behavior during the following 48 hour period as they ate their standard diet. We then reweighed all gulls and released them. Data for gull weights or number of earthworms consumed could not be normalized, so treatment and control groups were compared by Wilcoxon signed rank tests (significance was assessed at $P < 0.05$).

Field Application of Early Bird Fertilizer

On account of extended drought conditions, experiments intended to mimic field application of TSP (Early Bird Fertilizer) under ambient conditions were not possible at airports in northern Ohio or in a statistically adequate block design on PBS. However, we conducted a preliminary study using a single site characterized by Kibbie loam soil (Ernst and Martin 1994) to examine whether the number of *Lumbricus* and *Aporrectodea* spp. would be reduced in treated areas, and we report descriptive statistics only.

The selected site was an urban lawn with a mean grass height of 5.6 cm, which had not been managed with fertilizers or pesticides for the past 27 years and was located on a flat area, thus reducing the probability of movement of TSP leachate from one area to another. We established 18 circular 25 m^2 plots, each 8 m apart from the nearest plot. All plots were sampled on 13 May 2013, prior to application, by randomly tossing a square frame (0.125 m^2) into each plot and applying mustard-based expellant (Gunn 1992, Hale 2007) to verify earthworm presence. Early Bird Fertilizer has been formulated as granules containing N (3%) and soluble potash (1%) derived from tea-seed meal, kelp extract, and composted poultry litter, and as a liquid containing urea N (3%), tea seed extract (10%), and kelp extract (10%). Therefore we randomly selected 6 plots each to receive the granules, liquid formulation, or only water. On 15 May 2013, we applied TSP granular at a rate of 2.93 kg/100 m^2 or liquid formulation at a rate of 55 L/ha. After application of treatments, we simulated rainfall of about 1.25 cm within each plot by watering all plots with a sprinkler using water from a dug well. About 24 hr after treatment, we sampled for dead worms on the surface by randomly placing a square frame (0.125 m^2) within each plot, and counting and identifying all dead worms to genus (Hale 2007). All plots were resampled by mustard extraction, as described above, 1 week after treatment. We identified earthworms to genus and counted them. Above-average temperatures and below-average rainfall over the following 4 weeks greatly suppressed earthworm activity, so no further sampling was done until 23 September 2013 (17 weeks after treatment) when surface activity of earthworms within the area had resumed. We again sampled all plots by mustard extraction, as before, but this time within a 1 m^2 area in the center of each plot.

RESULTS

Simulated Runway Experiments

Light readings showed no illumination bias between treated and control tables ($P \geq 0.62$). Worms used all quadrants equally on the control tables (Table 2). Fewer worms used the treated quadrants compared with the untreated quadrants on the treatment tables for deer repellent and ammonium sulfate treatments (Table 2). However, when we compared the ratios of worm use on the treatment to worm use on the control tables, only ammonium sulfate fertilizer showed a significant deterrent effect (Table 2).

In the second simulated runway experiment, about twice as many holes, indicative of earthworm activity, were formed in untreated quadrants (23.1 ± 7.8) than in ammonium nitrate-treated quadrants (10.1 ± 7.4) of the treatment table. Hole numbers across the control table were similar (quadrants 1 and 4: 27.8 ± 13.0 holes; quadrants 2 and 3: 25.8 ± 10.7 holes). Table ($F_{1,10} = 4.2$, $P = 0.07$) and table-by-day interaction effects ($F_{3,30} = 2.5$, $P = 0.08$) were not significantly different.

Simulated Airport Applications

Pretreatment earthworm counts (mean $\pm$ SD) were similar for treated and control plots (16.5 ± 10.0 vs. 15.3 ± 8.1 for total worms, respectively [$U = 0.79$, $P = 0.43$]; 4.2 ± 3.9 vs. 3.8 ± 3.1 for *Lumbricus* spp. [$U = 0.12$, $P = 0.91$]; 12.3 ± 7.1 vs. 11.4 ± 6.2 for *Aporrectodea* spp. [$U = 0.78$, $P = 0.43$]). After 6 applications of ammonium sulfate over 2 years, the number of total earthworms (18.9 ± 16.9 vs. 38.8 ± 22.6 for treated and control plots, respectively) differed ($U = 2.34$ $P = 0.02$). Response varied by earthworm genus. Numbers ($U = 3.20$, $P < 0.01$) and mass ($U = 3.52$, $P < 0.01$) of *Aporrectodea* spp. were reduced by ammonium nitrate (10.2 ± 10.7 vs. 30.4 ± 17.7 earthworms; 2.1 ± 2.3 vs. 6.9 ± 4.5 g mass, in treated and control plots, respectively). In contrast, we found a similar number ($U = 0.05$, $P = 0.96$) and mass ($U = 0.55$, $P = 0.58$) of *Lumbricus* spp. in treated (8.7 ± 9.1 worms, 8.3 ± 13.3 g) and control plots (8.3 ± 8.6 worms, 6.6 ± 9.3 g).

The repeated applications of ammonium sulfate, as expected, significantly ($U = 4.02$, $P < 0.01$) acidified the soil; mean pH levels in treated and control plots as measured after the fifth application, were 4.9 ± 1.0 versus 6.4 ± 0.8, respectively.

Gull Exposure to TSP Application

Gulls showed no discernible adverse effects during the 48 hour after feeding on TSP-exposed earthworms. Mean numbers (SD) of earthworms consumed by gulls in pretreatment were similar ($U = 0.96$, $P = 0.97$) for the treatment (9.4 ± 4.5 worms) and control groups (10.3 ± 7.1 worms). Gulls consumed similar numbers of treated and untreated earthworms during the test period (14.6 ± 6.1 vs. 15.3 ± 4.2 worms/gull, respectively; $U = 0.99$, $P = 1.0$). Neither group showed significant weight change between capture and release (485 ± 33 vs. 491 ± 30 g, respectively, for the treatment group; 511 ± 46 vs. 484 ± 52 g for the control group, $P = 0.62$, 0.30, respectively). We did not see any avoidance of treated worms or regurgitation of worms, listlessness, or aberrant behavior by birds in either group during or after exposing birds to treated worms. The number of pieces of the maintenance diet consumed for each bird remained consistent prior to and after testing, because each bird ate all fish products and at least half of the remaining food.

Field Application of Early Bird Fertilizer

Pretreatment earthworm counts were similar for all plots (Fig. 2). From pretreatment to the 1 week after-treatment sample, counts from control plots decreased 31%, compared with 74% and 84% reduction in granular and liquid treated plots, respectively. The drop in the control plots was apparently driven by hot and dry conditions reducing the number of *Aporrectodea* spp. (54% fewer). However, the decrease in *Aporrectodea* spp. in treated plots was proportionately larger (82% in granular; 100% in liquid, compared

Table 2. Mean number (SD) of *Lumbricus terrestris* remaining in treated or control quadrants on treated and control tables following 60 min exposure to test substances in simulated runway trials. We conducted experiments on the National Aeronautics and Space Administration's Plum Brook Station, Ohio, USA, during 2007–2011.

		Treatment table					Control table					
		Treated quads		Untreated quads		Treated vs. untreated quads (*P*)	Quads 1 and 4		Quads 2 and 3		Quad 1,4 vs. quad 2,3 (*P*)	Treatment vs. control table (*P*)
Experiment[a]	kg/ha	$\bar{x}$	SD	$\bar{x}$	SD		$\bar{x}$	SD	$\bar{x}$	SD		
Ammonium sulfate I	857	2.0	1.1	10.2	2.3	0.00	8.5	1.5	7.0	1.4	0.25	0.00
Ammonium sulfate II[b]	857	1.2	1.2	9.5	3.4	0.00	8.5	1.5	7.0	2.0	0.34	0.00
Deer repellent	1,488	5.3	1.2	7.8	2.1	0.01	7.0	2.3	6.3	2.0	0.65	0.16
Coal slag low concentrate	11,013	9.7	3.9	5.8	4.0	0.27	7.0	1.7	6.2	1.2	0.39	0.26
Coal slag high concentrate	16,520	8.5	3.2	6.0	1.8	0.32	7.7	2.3	7.3	3.1	0.86	0.67

[a] Six replications using 16 worms/replication under lab conditions were completed for each treatment. Means between quadrants on a table were compared using paired *t*-test. The mean of the ratios of worm use of quadrants between the treated and control tables were compared using a 2-sample *t*-test with 5 df.
[b] Two tests were conducted with the second verifying the results of the first.

with pretreatment samples). *Lumbricus* spp. showed a slight decline in control plots (8%), but much larger declines in treated plots (69% granular; 63% liquid). We observed American robins (*Turdus migratorious*) feeding on treated plots immediately and up to 24 hr after treatment. We found a mean of 3.8 ($\pm$3.5) dead earthworms on the surface in granular-treated and 5.0 ($\pm$2.7) on liquid-treated plots. By the second (autumn) sampling period, 17 weeks after treatment, the total number of worms collected in all plots exceeded that of the pretreatment period in spring (control 23%, granular 30%, liquid 12%; Fig. 2).

DISCUSSION

Although earthworms (*Lumbricus terrestris*) were repelled by ammonium sulfate in our simulated runway experiments, 6 applications over 2 years of simulated airport applications failed to reduce their numbers or biomass in the field. We do not believe that this difference in results between the lab (where we repelled earthworms) and field (where earthworms moved away from the treatment) is due to physiological reasons, but to the ability of *Lumbricus* spp. to burrow to a depth beyond the impact of ammonium sulfate and withstand a wide range of pH levels (Edwards and Bohlen 1996). Others (Slater 1954, Potter et al. 1985, Ma et al. 1990, Richardson 1938, as cited in Edwards and Bohlen 1996) have found that long-term applications of acidifying fertilizers often reduce earthworm populations, but our results showing variable effectiveness against representatives of 2 common genera underscore the need to identify the species causing problems. Should immediate results not be required, use of ammonium sulfate to reduce overall earthworm numbers near runways might be effective. Nitrogen fertilization, however, can also augment the nutritional quality of grasses for caterpillars and other insects (e.g., Davidson and Potter 1995), which

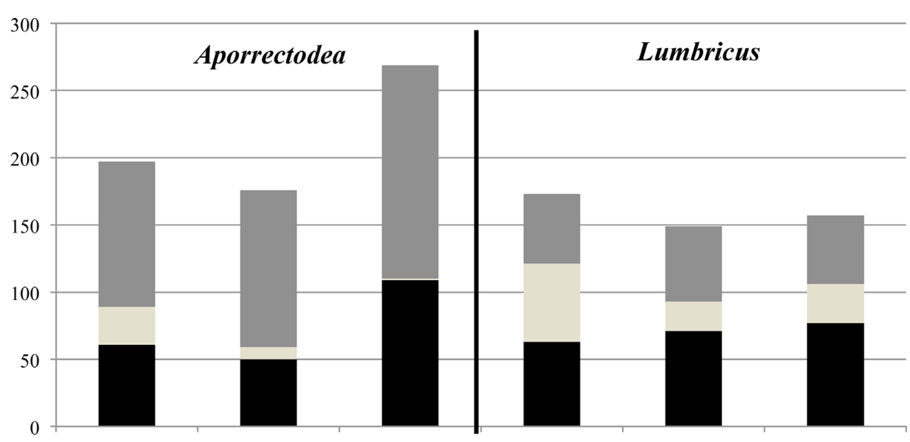

Figure 2. We conducted experiments on the National Aeronautics and Space Administration's Plum Brook Station, Ohio, USA, and at an area about 15 km south of this site, to identify products that could be used to repel or suppress nonnative earthworms on airports or other managed sites. Total number of *Aporrectodea* spp. and *Lumbricus* spp. removed via mustard extraction from 0.125 m^2 areas within 25 m^2 untreated plots ($N = 6$) or plots treated with the tea-seed cake pellet-based Early Bird fertilizer in granular (2.93 kg/100 m^2; $N = 6$) or liquid form (55 L/ha; $N = 6$) in Huron County, Ohio. Sample dates were 13 May, 22 May, and 23 September 2013 for pretreatment (Pretreat), and 1 and 17 weeks after treatment counts (Post), respectively.

could potentially translate to larger insect populations and attract additional birds.

Based on our preliminary study and acknowledging that we had limited replication we suggest that the tea-seed-based product Early Bird fertilizer shows promise for earthworm control because numbers of both *Lumbricus* spp. and *Aporrectodea* spp. decreased initially in treated plots. Additionally, our observations on declines in *Aporrectodea* spp. were similar to earlier reported results (Potter et al. 2010). Further, gulls that consumed the treated earthworms that normally die on the surface showed no reaction either to eating the earthworms or thereafter, and treated gulls maintained weight for 48 hour. Injured, sick, or stressed birds may use up substantial energy reserves that subsequently result in significant daily weight loss (Steiner and Davis 1981, Madej and Clay 1991, Wendeln and Becker 1996) and gulls in this study continued to feed and maintain body mass; therefore, we conclude that the TSP did not overtly impact gulls. We chose not to conduct more invasive testing of gulls because the toxicity of saponins is minimized when administered orally, in part because of their inability to cross the gut and enter into the bloodstream (Price et al. 1987).

On account of unseasonably hot and dry spring weather conditions, we were only able to assess reduction of earthworm numbers at 1 week after the spring (May) application before earthworms became seasonally inactive. This weather may also have added to the overall decrease we observed in earthworm numbers in treated sites; but because all sites were affected to the same extent, the observed changes are relevant, especially when compared with control plots. By September, earthworm populations in our treated plots had returned to pretreatment levels. Potter et al. (2010) demonstrated that a single TSP application reduced casting by *Aporrectodea* spp. for $\geq$5 weeks. Tea-seed saponins are likely to break down and dissipate in moist environments within a few days (Terazaki et al. 1980, Nagesh et al. 1999). Thus, more prolonged reductions in earthworm activity likely reflect the initial reduction in the population as opposed to extended residual activity. Mather and Christensen (1988) found that individual *L. terrestris* could move across the soil surface about 4–19 m in a single foray. Also, individual *L. terrestris* lay about 65 cocoons/adult, with each cocoon producing one juvenile, and cocoons may remain in the soil without hatching for up to 80 weeks (Daniel 1992). Individual *A. rosea* can produce 30–40 cocoons/year and *A. longa* can produce about 15 cocoons/year (Holmstrup 1999). Therefore, treated areas may be recolonized through hatching of cocoons as well as movement of earthworms into previously treated areas as temperature and moisture conditions permit (Edwards and Bohlen 1996). This would then necessitate treatment of areas of concern with TSP at least in the spring and autumn when worm activity is the highest.

A concern with TSP use on airports is that expelled earthworms may attract birds, which, based on our results, will readily consume the treated worms. This threat may be short-lived, (<24 hr) because emerging earthworms die on the surface and soon desiccate (Potter et al. 2010). If TSP could be applied at dusk, whereby earthworms come to the surface in the dark and are dead with initial drying occurring by dawn of the following day, bird attraction might be reduced. More likely, expelled dead and dying earthworms would continue to attract birds for some time, which could result in a temporary runway closure to remove the earthworms.

The commercial TSP-based fertilizer we used required about 1.5 cm of water to activate the saponins and leach them into the soil. Areas with walkways that have an irrigation system could follow a dusk application procedure. Most airports, however, would likely have to time TSP application with impending rainfall unless formulations are developed that remain field-stable until activated by rainfall.

A potential environmental concern related to rainstorm runoff of active TSP is the potential for a fish kill. When used at the relatively low dose rates of about 1.1 parts/million, saponin-based products derived from *Camellia* spp. have been used as agents to control unwanted predatory fish in shrimp ponds without affecting Crustacea (Tang 1961, Terazaki et al. 1980). However, in order to reach levels of TSP contamination that would impact aquatic life in a 3 m deep by 0.3 ha surface-area pond, 54 kg of tea-seed cake would be required (Potter et al. 2010). Therefore, potential aquatic issues could likely be addressed with appropriate label restrictions in regard to buffer zones around water and treatment rates.

Further research on earthworm control products should continue not only because of concerns at airports but also because of the impacts invasive earthworms have on ecosystem processes and functions in natural settings (Hendrix and Bohlen 2002; Bohlen et al. 2004 *a,b*; Hale et al. 2006; Keller et al. 2007). Once a population is established (whether on sport fields, airports, or in natural settings), control of invasive earthworms is difficult (Bohlen et al. 2004*b*, Callaham et al. 2006); therefore, research should not be limited to standard tools. For example, when stimulated, *L. terrestris* produces an alarm pheromone that is conspecific in repellency; the pheromone lasts for $\geq$3 months, but it may attract garter snakes (*Thamnophis sirtalis*) unless presented in a purified form (Ressler et al. 1968, Jiang et al. 1989). If costs for the production of this pheromone could be kept low and if it is efficacious, then it may provide another means of keeping earthworms from runways and deserves further scrutiny as a potential repellent. Should any product prove effective against earthworms, then researchers need to determine: 1) the likely period of earthworm reduction and how often an area must be treated; 2) the distance from runways or walkways to be treated to maintain the maximum benefit while reducing costs; and 3) whether other wildlife or native soil fauna are attracted or adversely affected.

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